Competitive Enzyme Immunoassay Kit for Quantitative analysis of Flumequine

1. Background

Flumequine is a member of the quinolone antibacterial, which is used as a very important anti infective in clinical veterinary and aquatic product for its broad spectrum, high efficiency, low toxicity and strong tissue penetration. It is also used for disease therapy, prevention and growth promotion. Because it can lead to drug resistance and the potential carcinogenicity ,the high limit of which inside the animal tissue has been prescribed in the EU, Japan(the high limit is 100ppb in the EU).

At present, spectrofluorometer, ELISA and HPLC are the main methods to detect flumequine residue, and ELISA has been a routine method for the high sensitivity and easy operation.

2. Test Principle

This kit is based on indirect-competitive ELISA technology. The microtiter wells are coated with coupling antigen. Flumequine residue in the sample competes with the antigen coated on the microtiter plate for the antibody. After the addition of enzyme labeled anti-antibody, TMB substrate is used to show the color. Absorbance of the sample is negatively related to the tetracycline reside in it, after comparing with the Standard Curve, multiplied by the dilution multiple, Flumequine residue quantity in the sample can be calculated.

3. Applications

This kit can be used in quantitative and qualitative analysis of flumequine residue in honey .

4. Cross-reactions

Flumequine …… 100%

5. Materials Required

5.1 Equipments

…… Microtiter plate spectrophotometer (450nm/630nm)

…… Homogenizer or stomacher

…… Shaker

…… Vortex mixer

…… Centrifuge

…… Analytical balance (inductance: 0.01g)

…… Graduated pipette: 15ml

…… Rubber pipette bulb

…… Polystyrene Centrifuge tube: 15ml, 50ml

…… Glass test tube：10ml

…… Micropipettes: 20μl-200μl, 100μl -10000μl, 250μl -multipipette

5.2 Reagents

-----n-hexane(AR)

-----Methylene chloride(AR)

-----Acetonitrile(AR)

-----Deionized water

-----Concentrated hydrochloric acid(AR)

6. Kit Components

- Microtiter plate with 96 wells coated with antigen
- Standard solutions(6 bottles×1ml/bottle)
  0ppb, 0.3ppb, 1.2ppb, 4.8ppb, 19.2ppb, 76.8ppb
- High concentration standard control:(1ml/bottle)
  ……**100ppb**
- Enzyme conjugate 12ml…….. red cap
- Antibody solution 7ml ……green cap
- Solution A 7ml……white cap
- Solution B 7ml …… red cap
- Stop solution 7ml ……yellow cap
- 20XConcentrated wash solution 40ml
  ……transparent cap
- 2X Extraction solution 50ml…… blue cap

7. Reagents Preparation

7.1 Honey sample

Solution 1 : 0.2 M Hydrochloric acid solution

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Weight 41.5ml Concentrated hydrochloric acid , dilute with deionized water to 500 ml.

Solution 2: Wash solution

Dilute the concentrated wash solution with deionized water in the volume ratio of 1:19,which will be used for washing the plates. the diluted solution can be stored at 4℃ for 1 month.

Solution 3: extraction solution

Dilute the 2×concentrated extraction solution with deionized water in the volume ration of 1:1 (or depend on requirement), which will be used for sample extraction. This diluted solution can be conserved for 1 month at 4℃.

8. Sample Preparations

8.1 Notice and precautions for the users before operation

(a) Please use one-off tips in the process of experiment, and change the tips when absorb different reagent.

(b) Make sure that all experimental instruments are clean , otherwise it will effect the assay result.

8.2 Honey sample

-----Weigh 2g±0.05g honey sample into a 50ml polystyrene centrifuge tube,

-----Add 2ml 0.2 M Hydrochloric acid solution (Solution 1), vortex to mix it completely, then add 8ml methylene chloride, shake with shaker for 5min to dissolve completely;

-----Centrifuge for 10 min, at least 3000g at room temperature (20-25℃);

-----Remove the supernatant phase, take 2 ml of the substrate organic solution to a 10 ml glass tube.dry the substate under water bath of Nitrogen flow (50-60℃)

-----Add 1 ml n-hexane,vortex for 30s, then add 1ml extraction solution(solution 3) ,vortex again for 1min. Centrifuge for 5min, at least 3000g at room temperature (20-25℃);

-----Remove the supernatant phase, take 50μl for assay;

9. Assay process

9.1 Notice before assay

9.1.1 Make sure all reagents and microwells are all at room temperature (20-25℃).

9.1.2 Return all the rest reagents to 2-8℃ immediately after used.

9.1.3 Washing the microwells correctly is an important step in the process of assay; it is the vital factor to the repetitiveness of the ELISA analysis.

9.1.4 Avoid the light and cover the microwells during incubation.

9.2 Assay Steps

9.2.1 Take all reagents out at room temperature (20-25℃) for more than 30min, homogenize before use.

9.2.2 Get the microwells needed out and return the rest into the zip-lock bag at 2-8℃ immediately.

9.2.3 The diluted wash solution should be rewarmed to be at room temperature before use.

9.2.4 **Number:** Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.

9.2.5 **Add standard solution/sample:** Add 50 μl of standard solution or prepared sample to corresponding wells. Add 50μl antibody solution. Mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover.

9.2.6 **Wash:** Remove the cover gently and pure the liquid out of the wells and rinse the microwells with 250μl diluted wash solution (solution 2) at interval of 10s for 4-5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tip).

9.2.8. **Enzyme conjugate:** Add enzyme conjugate solution 100μl to each well, Mix gently by shaking the plate manually and incubate for 30min at 25℃ with cover. Repeat the wash step again.

9.2.8 **Coloration:** Add 50μl solution A and 50μl solution B to each well. Mix gently by shaking the plate manually and incubate for 15 min at 25℃ with cover(see 12.8).

9.2.9 **Measure:** Add 50μl the stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450nm against an air blank (It's suggested measure with the dual-wavelength of 450/630nm. Read the result within 5min after addition of stop solution.) (We can also measure by sight without stop solution in short of the ELIASA instrument)

10. Results

10.1 Percentage absorbance

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbance (\%)} = \frac{B}{B0} \times 100\%$$

B ——absorbance standard (or sample)

B0 ——absorbance zero standard

10.2 Standard Curve

To draw a standard curve: Take the absorbance value of standards as y-axis, semi logarithmic of the concentration of the flumequine standards solution (ppb) as x-axis.

--- The **flumequine** concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution multiple of each sample followed, and the actual concentration of sample is obtained.

For data reduction of the ELISA kits, special software has been developed, which can be provided on request.

11. Sensitivity, accuracy and precision

Test Sensitivity： 0.3ppb

Honey Sample dilution factor: 2

Detection limit

Honey sample----------1ppb

Accuracy

Honey sample ---------- 90±20%

Precision

Variation coefficient of the ELISA kit is less than 10%.

12. Notice

12.1 The mean values of the absorbance values obtained for the standards and the samples will be reduced if the reagents and samples have not been regulated to room temperature (20-25℃).

12.2 Do not allow microwells to dry between steps to avoid unsuccessful repetitiveness and operate the next step immediately after tap the microwells holder.

12.3. Homogenize each reagent before using.

12.4. Keep your skin away from the stop solution for it is the 2M H_2SO_4 solution.

12.5 Don't use the kits out of date. Don't exchange the reagents of different batches, for it will drop the sensitivity.

12.6 Storage condition:

Keep the ELISA kits at 2-8℃,do not freeze. Seal rest microwell plates Avoid straight sunlight during all incubations. Covering the microtiter plates is recommended.

12.7 Indications for the reagents going bad:

Substrate solution should be abandoned if it turns colors.

The reagents may be turn bad if the absorbance value (450/630nm) of the zero standard is less than 0.5 (A450nm＜0.5).

12.8 The coloration reaction needs 15min after adding Solution A and Solution B. And you can prolong the incubation time ranges from 20min to more if the color is too light to be determined. Never exceed 25min, On the contrary, shorten the incubation time properly.

12.9 The optimal reaction temperature is 25℃. Higher or lower temperature will lead to the changes of sensitivity and absorbance values.

13. Storage

Storage condition: 2-8℃.

Storage period: 12 months.

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